# EFFECT OF MICROWAVES ON RED BLOOD CELL COMPONENTS: INVESTIGATIONS AT THE MOLECULAR LEVEL

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## ABSTRACT

Raman spectroscopy has been employed as a non-perturbing, specific and readily interpretable technique to probe the molecular effect of microwaves on intact erythrocytes (red blood cells) and erythrocyte ghosts (cell membranes). By excitation within the absorption band of hemoglobin, we have for the first time measured the resonance Raman spectrum of the hemoglobin bound within intact erythrocytes. Exposure to 2.4 GHz (CW) microwave radiation at power densities between 1 and 25 $mW/cm^2$ results in no discernible change in the hemoglobin spectrum. Resonance Raman spectra of heme proteins are known to be quite sensitive to changes in iron spin state, oxidation state and porphyrin ring conformation. Therefore, we conclude that none of these molecular-level factors is perturbed by microwave fields of the frequency and intensity employed here.

Conventional Raman spectra have been obtained from preparations of hemoglobin-free erythrocyte ghosts. Vibrational modes from both the protein and lipid components of the membrane have been identified. A systematic study of the temperature dependence of the relative intensities of the conformationally sensitive C-H stretching vibration shows a steep transition between ~35° and 40°C -- i.e. within the physiological temperature range. Investigation of the effect of microwave radiation on erythrocyte ghosts in this temperature regime will be reported.

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Investigations of the molecular effects of microwave radiation on biological materials requires a non-perturbing, specific, sensitive and readily interpretable technique. One such technique which has (in our hands) proven quite successful is laser Raman spectroscopy. Raman scattering may be observed from a sample without the necessity of adding exogenous probes and with absolutely no perturbation of the microwave environment. A Raman spectrum contains richly detailed information on the vibrational structure of the molecule under study. The application of the technique and interpretation of the data have proven to be of significant value for studies of the structure of biological macromolecules.(1,2)

Excitation at any wavelength and with moderate power will result in "conventional" Raman spectrum yielding a complete and often very complex spectrum. By contrast low-power excitation within the absorption band of a chromophoric molecule results in an intense, resonance Raman spectrum characterized by a small number of vibrational modes localized on the chromophore. Resonance Raman spectroscopy achieves the dual goal of high selectivity and significantly enhanced sensitivity, such that direct spectroscopic examination of a protein's active site becomes possible.(3) Heme-containing proteins such as hemoglobin, cytochrome-c, and cytochrome oxidase have been extensively studied by resonance Raman.(1) For these proteins, the resonance Raman spectra are found to be quite sensitive to changes in iron spin-state, exidation state and prophyrin ring conformation. The active site ligand of the iron protein transferrin was unequivocally identified by its resonance Raman spectrum.(3) More recently, we have applied the technique to determine the active-site ligand in the protein uteroferrin.(4) We have now extended the range of the technique to an examination of intact red blood cells in the absence and presence of microwave fields. As well, we have applied "conventional" Raman to a detailed vibrational study of red cell membranes.

Intact Erythrocytes

Fresh, packed blood cells (erythrocytes) (American National Red Cross Blood Research Laboratory) were washed twice by suspending them in 0.9% NaCl and centrifuging for 15 min at 2,500 rpm. A dilute (pink) suspension of the washed cells in KClO (200 mM) was placed in a melting point capillary and illuminated transversely using 20 mW of 5145 Å laser light. The resonance Raman spectrum (Fig. 1) so obtained is that of the heme moeity of the erythrocyte-contained hemoglobin. To our knowledge this is the first such spectrum from intact erythrocytes.

The capillary cell was placed in an air-thermostatted anechoic chamber. The temperature of the chamber was maintained at 37°C ± 0.15°C. CW microwave radiation was supplied to an octave bandwidth horn suspended above the sample cell. Power densities at the sample site were measured by a miniaturized dipole antenna probe developed by BRH. The blood cell sample was irradiated with CW microwave radiation at various power densities between 1 and 25 mW/cm$^2$ and 2.4 GHz frequency. At each power level chosen, a resonance Raman spectrum of the cell suspension was recorded and analyzed. No discernable changes in the hemoglobin spectra were observed over the power range studied. Therefore, we conclude that no changes occur in iron spin-state, oxidation state or porphyrin ring conformation under the influence of microwave fields of the frequency and intensity employed here.

## Erythrocyte Ghosts

Erythrocyte ghosts (membranes) were prepared from fresh, packed erythrocytes using a slightly modified Dodge procedure.[5] One unit of packed red blood cells was washed as described above and lysed in a dilute buffer (Tris-HCl (0.005M) - EDTA (.001 M)). The ghosts were washed four times in Tris-EDTA by centrifugation at 13,000 rpm for 30 min. The hemoglobin-free pellets were pooled, suspended in 5 ml $H_2O$ (or $D_2O$) and sedimented 1 hour at 25,000 rpm in an SW 50.1 swinging bucket rotor (washing in $D_2O$ resulted in a substantial improvement in Raman background). Finally the milk-white pellets were resuspended in 5 mm $NaHPO_4$ (ph 7.4) and spun-down at 25,000 rpm for 1 hr. "Conventional" Raman spectra of the ghosts were obtained from concentrated samples illuminated with 400 mW of 5145 Å light. In the spectral region from 700-1700 cm$^{-1}$ (Fig. 2) we are able to identify vibrational bands from both the protein and lipid components of the membrane.

Conformation-sensitive C-H stretching modes fall in the frequency region 2750-3100 cm$^{-1}$ (Fig. 3). A systematic study of the temperature dependence of the relative intensities of the C-H stretching modes shows a steep transition between 35° and 40°C -- i.e. within the physiological temperature range. Investigation of the effect of microwave radiation on erythrocyte ghosts in this temperature regime is now underway and will be reported.

## Acknowledgement

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RESONANCE RAMAN SPECTRUM OF HEMOGLOBIN IN INTACT ERYTHROCYTES

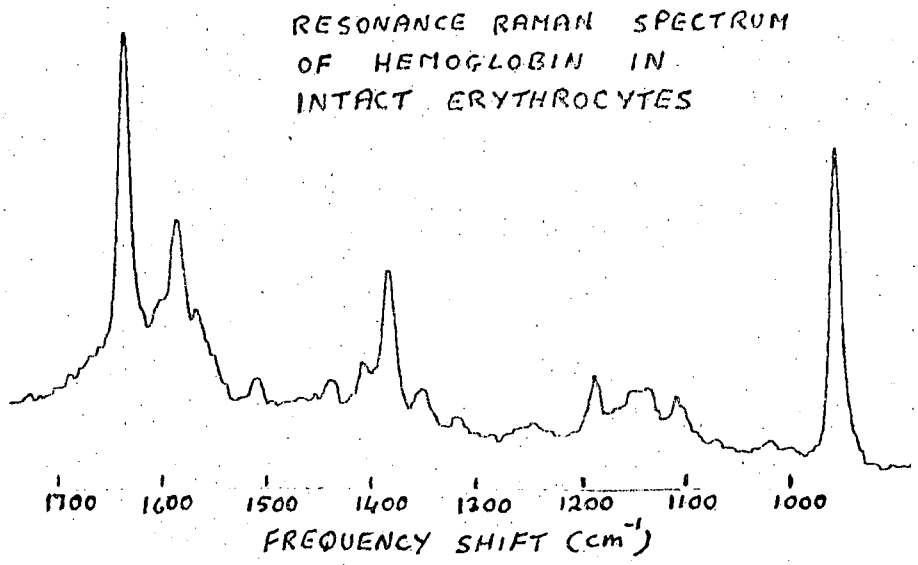
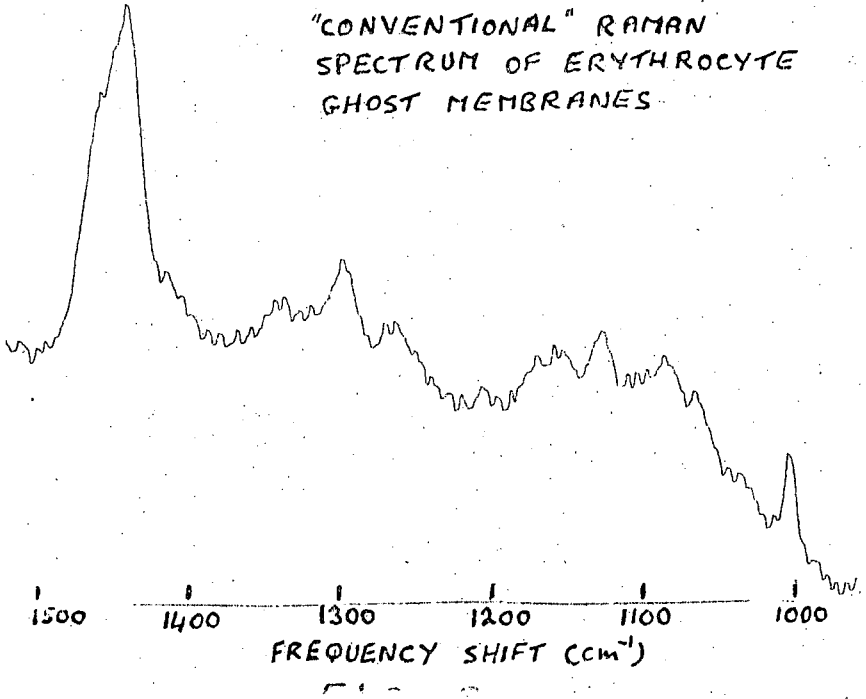



FIG. I

"CONVENTIONAL" RAMAN SPECTRUM OF ERYTHROCYTE GHOST MEMBRANES




ERYTHROCYTE GHOST SPECTRUM: C-H STRETCHING REGION

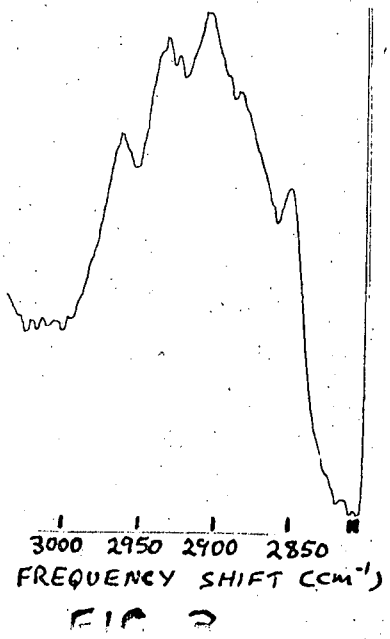



FIG 2